# Turnover of some biochemical constituents during embryogenesis of *Antheraea mylitta* Drury to monitor the efficacy of carbendazim and chloroquine in controlling microsporidiosis

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**Abstract.** The microsporidian *Nosema* sp. is a major pathogen of the tropical tasar silkworm, *Antheraea mylitta* Drury. In acute condition, the disease affects moth emergence, reproductive potential, egg and larval viability, and cocoon characters. Efficacy of 0.005% carbendazim (a systemic fungicide) and 0.50% chloroquine (an anti- protozoan drug) on the virulence of *Nosema* sp. was investigated through studies of the 24-hourly turnover of total proteins, total carbohydrates, total lipids and total free amino acids during embryogenesis of *A. mylitta*. Results of this experiment indicate that chloroquine appeared less deleterious to *Nosema* sp. in comparison to carbendazim which was found to be significantly effective in restoring the level of all four biochemical constituents. Also, the effectiveness of carbendazim was reflected in a significant improvement of the effective rate of rearing and silk ratio while chloroquine was least effective.

## Introduction

Microsporidians are generally considered a most important group of protozoan parasites infecting insects (McLaughlin, 1971). Pebrine, caused by the microsporidian, *Nosema* sp., is an important mortality factor of tasar silkworm, *Antheraea mylitta* Drury . It has threatened both tasar culture and tasar seed production. The pathogen is transmitted both horizontally and vertically in the population and multiplies rapidly (Jolly & Sen, 1972). Further, outdoor rearing of tasar silkworm enhances the possibility of the pathogen being disseminated through common vectors, making it extremely difficult to eradicate the disease. Various attempts have been made to suppress the microsporidian infection in insects (Allen & Brunson, 1949; Katznelson & Jamieson, 1952; Bailey, 1953; Jamieson, 1955; Fox & Weiser, 1959; Weiser, 1961; Moffett *et al.*, 1969; Lynch & Lewis, 1971; Wilson, 1974; Hamm *et al.* 1977; Xian, 1987), but a suitable therapy has not yet been discovered.

Effectiveness of fungicides to control the disease has been investigated by several workers (Flint *et al.*, 1972; Hsiao & Hsiao, 1973; Armstrong,

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1976; Harvey & Gaudet, 1977; Brooks *et al.*, 1978 and Griyaghey *et al.*, 1987). Chloroquine, mainly an antimalarial drug, was found effective against other protozoan infection such as *Trypanosoma* (Otigbuo & Patrick, 1988). Consequently the present study was undertaken to test various doses of a systemic fungicide, carbendazim, { 2(Methoxy - carbomylamino) Benzimidazole} and an antiprotozoan drug, chloroquine. Efficacy of carbendazim and chloroquine was monitored throughout embryogenesis by measuring 24-hourly turnover of proteins, carbohydrates, lipids and free amino acids. These biochemical constituents were reported to decrease in eggs following infection (Sinha *et al.*, 1988 and Sinha *et al., 1991* ).

## Materials and Methods

Larval stages were treated with drugs during rearing. Assay larvae were reared in four batches with *Terminalia arjuna* as host plant. The first batch comprised of healthy larvae (uninfected control) was reared in a separate field to avoid secondary contamination. The other three batches were pebrine infected and comprised an infected control, carbendazim treated and chloroquine treated. These larvae were reared at different locations in one field. The third and fourth batches were treated with 4 concentrations of carbendazim (0.005, 0.01, 0.02 and 0.04%) and 3 concentrations of chloroquine (0.01, 0.1 and 0.5%) respectively. The doses were selected on the basis of exploratory trials in previous years. Drug administration was by foliar spray of an aqueous solution using a Knapsack sprayer.

Carbendazim was fed continuously to the larvae from II stage onward until the cocoon was spun. Chloroquine was fed for 3 days to II and III stage larvae respectively. Five replicates of 200 larvae each were initially taken per batch for all treatments. The mortality of larvae was recorded regularly and the data were statistically analyzed.

The treatment which showed the best rearing performance, 0.005% carbendazim (CB) and 0.50% of chloroquine (CQ), was chosen for the biochemical assays throughout embryogenesis at 24-hour intervals. Four biochemical constituents, viz. total proteins, total carbohydrates, total lipids and total free amino acids were assayed by the methods of Lowry *et al.* (1951), Dubois *et al.* (1956), van Handel (1985) and Moore and Stein (1948) respectively.

## Results and Discussion

Data presented in Table1 indicate rearing performance of carbendazim (CB) and chloroquine (CQ) in controlling the micosporidiosis. It is evident that CB at 0.005% is significantly ($P< 0.05$) effective in increasing survivability (ERR) and silk ratio in comparison to the infected, but untreated control set. Increasing CB concentration does not significantly decrease larval mortality due to pebrine. However, the economic characters of ERR & silk ratio decrease significantly. The data suggest a side effect of higher doses of CB on the larvae of A. *mylitta*. The effect of 0.50% CQ is superior to other doses of this drug for ERR and silk ratio. However, when performance of both the drugs are compared vis-a-vis economic yield, CB has an edge over CQ.

Table 1. Rearing performances of carbendazim and chloroquine in controlling the microspordiosis.

| Drug | Concentration (%) | LMM (%) | ERR (%) | Cocoon Characters C.W. (g) | S.W. (g) | Silk Ratio (%) |
|---|---|---|---|---|---|---|
| Carbendazim | 0. 005 | 31.10 | 45. 60 | 10. 80 | 1.29 | 11.94 |
| | 0. 010 | 28.20 | 27. 00 | 11. 00 | 1.15 | 10.41 |
| | 0. 020 | 27.60 | 28.60 | 10.47 | 0.88 | 8.36 |
| | 0. 040 | 26.60 | 26. 50 | 11.21 | 1.29 | 11.50 |
| | Infected control | 60.00 | 19. 50 | 10.89 | 1.10 | 10.08 |
| | CD at 5% | 4.114 | 15.333 | 1.072 | 0.223 | 1.587 |
| Chloroquine | 0.010 | 38.10 | 27.90 | 11.29 | 1.28 | 11.36 |
| | 0.100 | 34.80 | 24.80 | 11.20 | 1.21 | 10.83 |
| | 0.500 | 31.00 | 30.10 | 11.20 | 1.31 | 11.72 |
| | Infected control | 60.00 | 19.50 | 10.89 | 1.10 | 10.08 |
| | CD at 5% | 5.982 | 7.677 | 0.477 | 0.157 | 1.239 |
| | Healthy control | 0.00 | 47.20 | 12.24 | 1.32 | 10.75 |
| | Pooled CD at 5% | 13.071 | 17.388 | 0.670 | 0.205 | 1.735 |

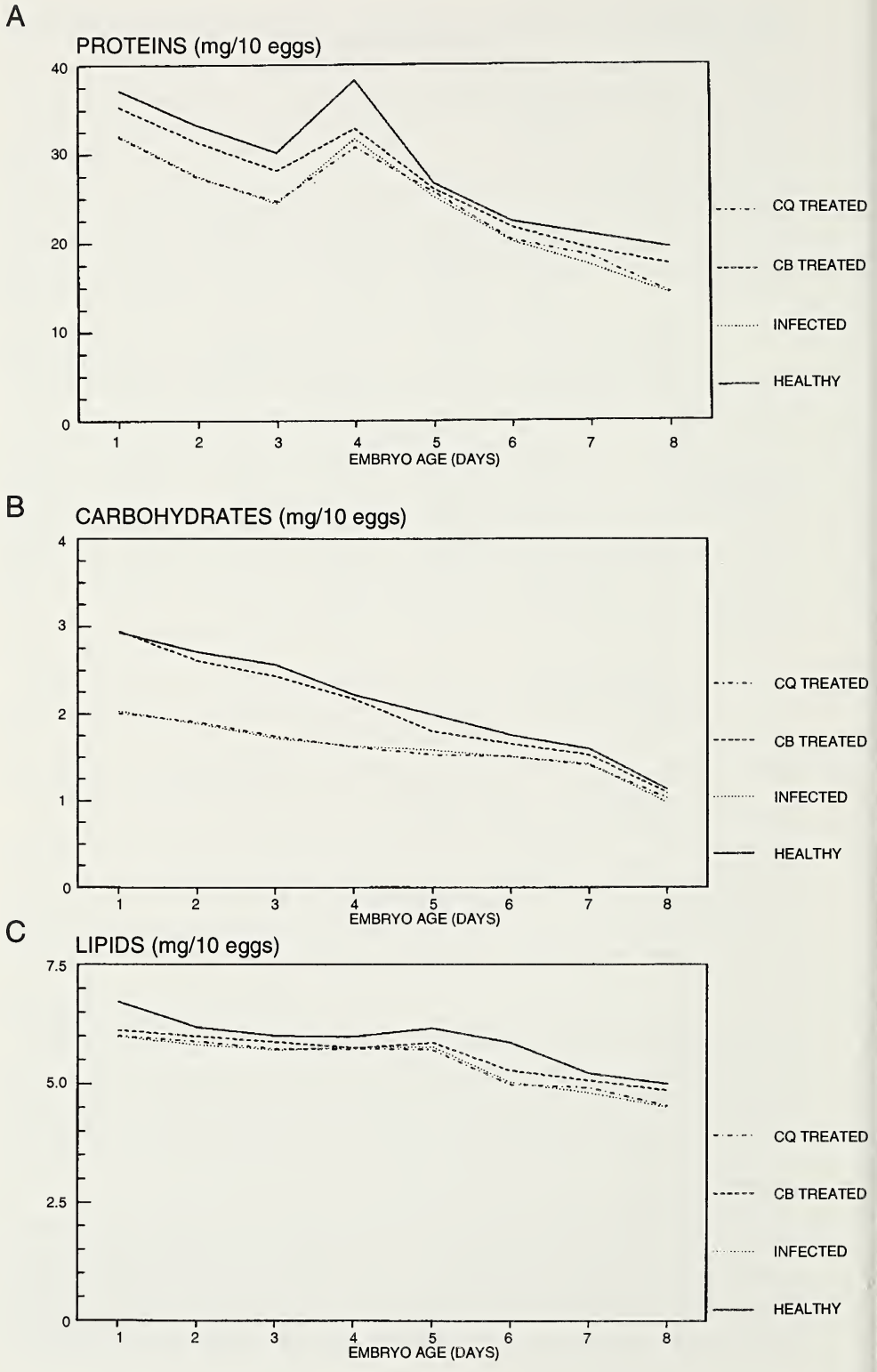
LMM - Larval mortality due to microsporidiosis; ERR - Effective rate of rearing; C. W. - Cocoon weight; S. W. - Shell weight .

## Biochemical monitoring

Daily changes in healthy, pebrine infected, CB (0.005%) treated and CQ (0.50%) treated eggs of *A. mylitta* with regard to total proteins, total carbohydrates, total lipids and total free amino acids are given in Figures 1a, b, c & d respectively. The data were recorded at 24-hour intervals and during the complete period of embryogenesis until hatching . Figures 1a to 1d reveal that all four biochemical parameters show an almost uniform pattern of rise and depression with respect to all treatments: healthy, infected, CB treated and CQ treated. The data obtained during embryogenesis are pooled and presented in Table II.

## Total Proteins

As evident from Fig. 1a, the concentration of proteins in all four treatments decreases from the first to the third day of embryogenesis and then increases on fourth day, finally decreasing until the larvae hatch. Rise and fall in protein level during embryonic development suggest both breakdown and synthesis of organ specific proteins occur-


A
PROTEINS (mg/10 eggs)
40
30
20
10
0
1
2
3
4
5
6
7
8
EMBRYO AGE (DAYS)
CQ TREATED
CB TREATED
INFECTED
HEALTHY
B
CARBOHYDRATES (mg/10 eggs)
4
3
2
1
0
1
2
3
4
5
6
7
8
EMBRYO AGE (DAYS)
CQ TREATED
CB TREATED
INFECTED
HEALTHY
C
LIPIDS (mg/10 eggs)
7.5
5.0
2.5
0
1
2
3
4
5
6
7
8
EMBRYO AGE (DAYS)
CQ TREATED
CB TREATED
INFECTED
HEALTHY

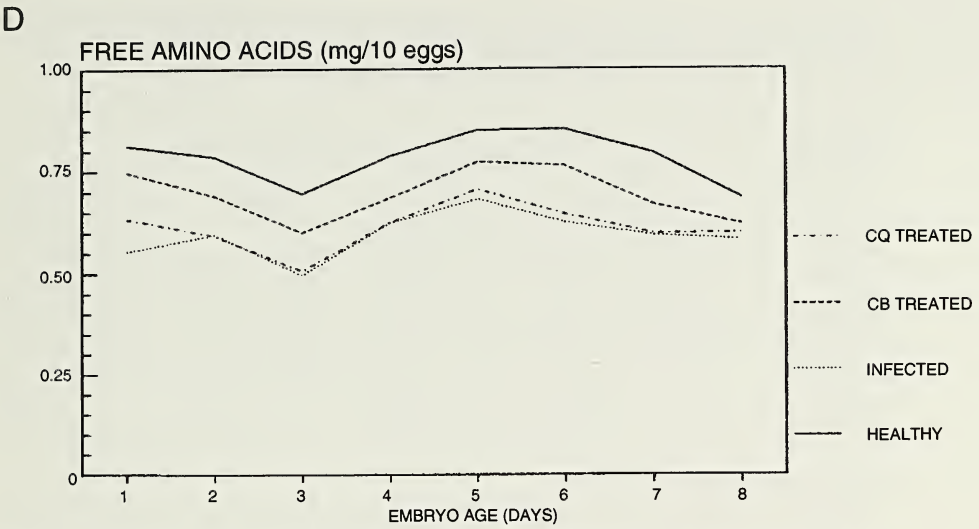



Fig. 1. Showing 24-hourly turnover in the concentrations (mg/10 eggs) of biochemical contituents during embryogenesis of A. mylitta D. with respect to four treatments viz. healthy, infected, carbendazim (CB) and chloroquine (CQ).
a. Changes in the concentration of total proteins
b. Changes in the concentration of total carbohydrates.
c. Changes in the concentration of total lipids.
d. Changes in the concentration of total free amino acids.

ring simultaneously (Sinha *et al.*, 1991). The data of Table 2 indicate that CQ has no effect while CB is significantly ($P< 0.05$) effective in improving the protein level in comparison to infected animals.

**Total Carbohydrates**

Total carbohydrates show a gradual decrease in concentration during the course of embryogenesis in all the four treatments (Fig. 1b). Urbani and Bellini (1959) observed a gradual decrease in carbohydrates for energy requirements during embryogenesis of silkworm *Bombyx mori*. Sinha *et al.* .(1991) reported similar trends of turnover in healthy and pebrine infected eggs of *A. mylitta* during embryogenesis. Table 2 indicates that CB treatment significantly restores the level of carbohydrates of infected embryos while CQ has no effect.

**Total Lipids**

Fig. 1c shows that total lipids also fall gradually from first day till the termination of embryogenesis, except for a slight rise on day 5. Our study corroborates the report of Goel *et al.* (1988) for the developing healthy embryos of *A. mylitta*. Table 2 shows that CB has significant ($P< 0.05$) impact on restoring lipid levels in comparison to the values of infected eggs. CQ was again ineffective.

Table 2. Effect of carbendazim (CB) and chloroquine (CQ) on pooled values of total proteins, total carbohydrates, total lipids and total free amino acids in the eggs (Mean ± Standard Error)

| Treatments | Biochemical Constituents (mg/10 eggs) | | | |
|---|---|---|---|---|
| | Total Proteins | Total Carbohydrates | Total Lipids | Total Free Amino Acids |
| Healthy | 28.525 ± 1.428 | 2.111 ± 0.120 | 5.894 ± 0.108 | 0.780 ± 0.012 |
| Infected | 24.067 ± 1.258 | 1.590 ± 0.062 | 5.431 ± 0.108 | 0.594 ± 0.011 |
| CB treated | 26.513 ± 1.278 | 2.027 ± 0.121 | 5.600 ± 0.092 | 0.693 ± 0.013 |
| CQ treated | 24.175 ± 1.187 | 1.591 ± 0.060 | 5.437 ± 1.106 | 0.613 ± 0.012 |
| CD at 5% | 0.234 | 0.018 | 0.013 | 0.006 |

**Total Free Amino Acids**

Figure 1d shows the turnover of free amino acids during the embryogenesis for all the four treatments. The trend of change in free amino acids content in healthy and infected eggs confirm the observations of Sinha *et al.* (1988) in *A. mylitta*. The data of Table 2 indicate that CQ correlates with CB to significantly ($P < 0.05$) increase the concentration of total free amino acids in infected eggs. The efficacy of chloroquine may be attributed to its retardation of protein synthesis in protozoa (Vial *et al., 1988*), thereby raising the free amino acid pool.

The results of this study suggest that 0.005% carbendazim treatment of larval stages during rearing has a definite effect in suppressing the development of *Nosema* sp. in *A. mylitta* while 0.50% chloroquine has the least effect. Hsiao & Hsiao (1973) demonstrated that Benomyl, another systemic fungicide, is an antimicrosporidian agent on a *Nosema* sp. in the alfalfa weevil. In fact, Benomyl containing diets fed to parasitized weevils for three days completely eliminated the *Nosema* parasite. Shinholster (1974), Armstrong (1976) and Harvey & Gaudet (1977) also observed the effectiveness of Benomyl against microsporidian infection. Griyaghey *et al.* (1987) experimented with treating eggs and larvae of *A. mylitta* with 2% Bengard (a systemic fungicide ) and observed decreased concentrations of spores in infected larvae. This ultimately increased the vigor and viability of larvae and produced higher yields and silk content. All these findings concur with our present results. Thus the biochemical parameters employed in our study may be useful as tools to monitor the efficacy of other drugs. In order to understand the mode of action of these drugs with the biochemical constituents, further experiments are necessary.

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